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W.S.PATTON

Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India.

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SCIENTIFIC MEMOIRS

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OF THE

GOVERNMENT OF INDIA

PRELIMINARY REPORT ON AN INVESTIGATION INTO THE ETIOLOGY OF ORIENTAL SORE IN CAMBAY

BY

CAPTAIN W. S. PATTON, M.B., I.M.S.

Offg. Director, King Institute of Preventive Medicine, Madras.

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PRELIMINARY REPORT ON AN INVESTIGATION INTO THE ETIOLOGY OF ORIENTAL SORE IN CAMBAY.

IN June 1910 I was placed on special duty, in order to investigate the etiology of Oriental Sore in India, and on learning from Dr. Row that this well known skin disease is endemic in Cambay, I went there in order to study it under the most favourable conditions; the main object of my enquiry was to try and find out exactly how the parasite of Oriental Sore is transmitted in nature. I spent in all six and a half months in Cambay and during that time saw about sixty typical cases, and was able to collect a large amount of valuable information regarding the etiology of the disease. As I hope in due course to write a complete account of my investigations, describing the disease as I saw it in Cambay, as well as the life history of the parasite, I do not propose in this paper giving any great detail, but merely recording my feeding experiments, so that others who are working at this, or allied parasites, may avail themselves of the results of my work.

Oriental Sore is said to have existed in Cambay for the last 250 years and probably was introduced from Persia; it is definitely localised to the town itself and hardly ever occurs among the villagers living in the surrounding country, except among those who make a short or prolonged stay in the town of Cambay. It was extremely difficult to obtain any accurate information, as to whether the disease shewed any tendency to spread among the villagers when introduced by infected people coming from Cambay. Recently Major Liston, I.M.S., saw a typical case of Oriental Sore in a girl in Bombay, who had never been to Cambay, but had resided in Gogo, a seaport town opposite Cambay, which holds much communication with it. This case clearly shews that the disease has a tendency to spread outside the endemic area.

During my stay in Cambay I lived in the dâk bungalow, a small house kindly lent me by His Highness the Nawab. It was situated just on the outskirts of the town, about a quarter of a mile distant, and close to it was the railway station. It is important to note that no one living in this locality contracted the disease although it was so near the town.

The day after my arrival in Cambay, I visited the local schools and selected a suitable case for experiments, a boy aged 10 who had a large fresh sore situated on the anterior surface of the lower third of his left thigh, which on examination was found to be eminently suitable for experimental purposes.

This case was observed daily for three and a half months, and during that time a very striking and suggestive observation was made, and one which was of frequent occurrence in the opinion of the people themselves. On several occasions the little boy informed me that his sore was swollen that morning, and on examining it, the margins were found to be more puffy than usual, and more marked on one or other side. On puncturing the swollen margin, I found there had been a marked extension of the parasitic area, and that the large macrophages were loaded with parasites; many of them had obviously ruptured as both the large mononuclear and polymorphonuclear leucocytes contained parasites. On the first occasion in which I observed this curious phenomenon I found two parasites in a large mononuclear leucocyte in a drop of blood taken from the upper third of the same thigh. On three other occasions parasites were found in the surface blood, taken a considerable distance from the lesion. This periodical swelling of the sore, followed by a marked extension of the parasitic area, resulting in the liberation of numbers of parasites into the circulating blood, at once recalls a very similar condition seen in Kala Azar. It will be remembered that I have pointed out that in that disease there are definite occasions when there is a great increase in the number of parasites found in the peripheral blood, and that these occasions correspond exactly with the extension of the parasitic area in the intestinal tract, as is clearly evidenced by the frequent onset of short dysenteric exacerbations. It was also found that more than half the cases of Oriental Sore in Cambay were totally unsuitable for purposes of experimental transmission of the parasite, for owing to bacterial invasion, chiefly staphylococci; the parasitic area is rapidly destroyed; this being clearly demonstrated in many cases which I had the opportunity of examining. Owing to the crude method of treatment,—in many cases no treatment at all,—the sore breaks down, and the parasites quickly disappear. Dr. Row in a recent paper read before the Bombay branch of the British Medical Association, states that Oriental Sore runs an obstinate course for months, and that parasites persist in the ulcers to the very end. This is entirely contrary to my observations, which could not have been better demonstrated than in my own sore. It began on January 9th, and in July the parasites had completely disappeared; the sore itself is still (October 1911) practically unchanged and is freely discharging and shews no tendency to heal. In Cambay I saw a large number of sores which I was able to observe for long periods, and found that the parasites disappeared a short time after the skin had broken. For feeding experiments I therefore consider the selection of a case of the utmost importance. The best cases are those, in which the sore does not break down, but in which the skin becomes covered with scales and scabs, and

except for a few small perforations, remains intact almost to the termination of the disease. It is in this type of sore that the periodical swellings caused by the marginal extension of the parasites are observed, and it is in these cases alone that the parasite can be found with any certainty in the circulating blood. Any one who has once seen this variety of sore, will not fail to recognise it again; I had the good fortune to see several during my stay in Cambay.

Having definitely settled that the parasite is frequently liberated into the circulating blood, and finding that the case under observation often exhibited this phenomenon, I utilised it for my feeding experiments. Dr. Row, who has on several occasions visited Cambay, has come to the conclusion that the house fly is the most probable transmitter. It was therefore important to settle this question once and for all. That house flies may transmit the parasite of Oriental Sore is no new hypothesis, for Seriziat and Laveran have long ago suggested this possibility. Laveran states, that from September to October at Biskra the slightest wounds tend to become transformed into true Oriental Sores and he adds that he has seen it grafted on to acne, impertigo, and vaccine pustules, as well as on wounds following burns, he therefore does not doubt that the virus is carried on the feet and proboscides of flies.

In this connection it should be remembered that these statements were made long before the parasite of Oriental Sore was discovered, and that many of the older observers have undoubtedly included, in their descriptions, sores other than the true specific lesion, such as are extremely common on the skins of the inhabitants of Eastern countries. When I was in the Aden Hinterland, and particularly at Sheik Othman, I examined many such sores thinking they were true Oriental boils, but never once found the characteristic parasite though many of these sores were exactly similar to the true "Bouton d' Orient." These ulcers and sores almost always contained spirochætes. Precisely the same state of affairs was seen in Cambay, for during the rainy season, from June to September, I found that the vast majority of skin lesions were not the true "Cambay Boil." As I was working in a room in the Hospital, I daily saw all the people who came for treatment, and sores and ulcers were invariably shewn to me. In this way I had the opportunity of examining a great many cases, but in the majority I failed to find any parasites.

On comparing the stained films made from these sores, with some made from the late stage of the true Oriental Boil, I was able to say at once whether these sores were ordinary ulcers, or whether they were the true lesion from which the parasite had disappeared. I will describe the characteristics of the latter in the complete account of my investigations. In Cambay I saw a few sores caused by blister beetles (*Cantharinæ*), several species of which were extremely prevalent during the rainy season. These insects being attracted by

lights, on entering a house are frequently crushed by the occupants lying on them, and the resulting blisters soon become chronic sores with indurated margins and closely simulate the true Cambay Boil.

As far as I am aware, no observer has as yet carried out exact feeding experiments with bred house flies in the endemic area, with a view to proving or disproving the fly transmission hypothesis. In my studies on the herpetomonads of the house flies of Madras I had already mastered the best methods for breeding these insects and keeping them in captivity. During June, July and August in Cambay, *Musca nebulo* Fabr. and *Musca sp.* were extremely abundant, and I do not recollect having seen these insects in such large numbers and constituting such a pest, in any part of India which I have visited. While driving from the dâk bungalow to the Hospital where I conducted my experiments, they followed me in large numbers, and in the Hospital itself they were present in swarms; so much so, that it was difficult to carry on one's work.

EXPERIMENT I.

Can the house fly carry the parasites on its legs (pulvilli) or its proboscis, from a sore discharging parasites, and deposit them on a scratch or abrasion on the skin of a susceptible person?

This experiment was carried out on my own person; several scratches and abrasions were made on the dorsum of my left hand, and flies were caught which had already crawled over and sucked up the whitish discharge from the sore mentioned above, and which was teeming with apparently living parasites. They were then allowed to crawl over and suck up the serum from the scratches on my hand. This experiment was carried out daily for about a month, and it was impossible to prevent the experiment under consideration being also carried out naturally, not only on my left hand, but on the right, and more particularly on my face; small cuts such as are commonly caused in shaving were constantly being contaminated by flies which had previously been on the sore. In spite of all these attempts, however, I never developed a single lesion on any of the experimental spots, and now more than a year has elapsed since they were carried out. Such a series of experiments, conducted in the endemic area, and under the most favourable conditions, conclusively prove that the house fly does not carry the parasite in the way mentioned above. In support of these results, there are the statements of the people of Cambay, who, although they have very crude ideas of the disease and its origin, have no hesitation in saying that the sore never begins in an open cut, scratch or abrasion, but always in a sound portion of skin.

In favour of the fly transmission hypothesis there is the undoubted fact that people, particularly children, who have a sore discharging parasites, frequently reinfect themselves by scratching the scab or the surroundings of the sore, and then scratching some other healthy part of the skin. The case I was studying thus reinfected himself, and it is interesting to note that at the time he had a great deal of prickly heat, and inoculated three places by scratching them with infected nails. When the rationale of this auto-infection is carefully considered, it will be seen that it is exactly similar to vaccination with cow-pox, for the parasite is deliberately inserted into the skin in the act of scratching. This method of auto-infection is extremely common, and the Jews in Baghdad and other parts of the world, have long practised it; recognising the readiness with which a sore can be produced on any part of the body they select a place where the resulting scar will cause no disfigurement.

Several experiments were carried out to try and see whether an already existing sore, (not the specific lesion) will become infected by placing some of the whitish discharge containing parasites on the surface of the granulations, such as a fly may do in the act of crawling over it. The experiments were carried out on three susceptible people who had recently come to Cambay, and in each case they failed. Lastly, a large number of sores, ulcers, scratches and abrasions in children, who had up till then not contracted the disease, were examined for months, but I was never able to find a single parasite in any of these sores. I consider this observation to be of importance, for it refutes the numerous loose statements, that during the fly season, when the sores are most prevalent, such cuts, abrasions, and ulcers are very liable to become infected with the parasite. These experiments, then, not only prove that the house fly is not a carrier in the way suggested, but that simple sores and ulcers which are so common, and always present on Indians, never become infected.

EXPERIMENT II.

DOES THE PARASITE WHEN INGESTED BY THE HOUSE FLY UNDERGO ANY DEVELOPMENT, OR DOES IT REMAIN LONG ENOUGH UNCHANGED IN ITS ALIMENTARY TRACT, SO THAT LATER IT MAY BE PASSED OUT IN ITS EXCRETA ON TO THE SKIN OF A SUSCEPTIBLE PERSON?

In order to carry out this experiment, flies (*Musca nebulo* and *Musca sp.*) were bred out from larvæ and kept in suitable cages; they were then taken out in small numbers in large glass tubes, and allowed to feed on the sore, just at the time when it was discharging parasites. They were next placed in a clean jar and examined at regular intervals; many of the flies were repeatedly fed.

Their alimentary tracts were dissected out in sterile salt solution and each separate portion was isolated and smeared out, and then stained by Romanowsky's stain. It was found that the parasites could be recovered only in the midgut, and unchanged, up to six hours after they were ingested, but that after that time they degenerated and disappeared. Most careful search was made in the hind part of the fly's intestine, particularly the rectum, but I was never able to recover a single parasite. These experiments clearly prove that though the fly undoubtedly often ingests parasites, they never undergo any development in its alimentary tract, but on the contrary, they soon degenerate as shewn in the smears when stained by Romanowsky's stain. A large number of wild flies were next examined, more particularly those which had already fed on the sore, but here again the parasites were never seen after six hours; the wild flies in Cambay were almost all heavily infected with herpetomonads and bacteria. The second experiment therefore proves, that in Cambay the alimentary tract of the house fly is not a suitable medium for the parasite, and that it never develops into a flagellate, but soon disintegrates and disappears. The results of these experiments are directly opposed to those of Row and to some recent observations made by Cardamatis and Melissidis who believe that the house fly is the invertebrate host of the parasite of Oriental Sore. Row, in his most recent communication regarding the method of transmission of the parasite, still adheres to the house fly (species not named) as the probable transmitter; he now however admits that the parasite soon disappears from the alimentary tract of a fly, an observation which I made more than a year ago in Cambay. This, in my opinion, definitely proves that the fly cannot infect another person by its excreta. He now pins his faith to the transmission of the parasite up to three hours after it has been ingested, and suggests that a fly so infected is capable of passing out living parasites, and that these may be deposited on a cut or abrasion or scratched in to the skin. He believes his view is proved by the fact that he has succeeded in infecting a monkey with the contents of the midgut of a fly which had ingested infected material three hours previously. I may, however, point out that this is no proof that the fly acts as the natural carrier: Basile has carried out a very similar experiment with the dog flea and the parasite of dog Kala Azar. Such an experiment merely indicates that the parasites remain alive, after having been three hours in the stomach of the fly, (which in this case was a clean insect) and is analogous to keeping the infected material in a test tube. In order that the transmission may take place, in the manner now suggested by Row, it is necessary for the flies to have clean alimentary tracts, whereas the wild flies in Cambay, judging from the contents of their stomachs, live almost entirely on filth. One of the

most convincing arguments against the fly transmission hypothesis, is the fact that although flies were most abundant during the rainy season in Cambay, I never saw a single early case which I could say was definitely infected during this time. From October to January I regularly examined some eight hundred children in three of the local schools, and although a number of them had not yet contracted the disease, I failed to find a single early lesion. The skin of each child was thoroughly searched, and any suspicious pimple or nodule was punctured and the contents microscopically examined. Further, if the transmission is regularly carried out by the fly, as suggested by Row, surely I would myself have become infected, for all the factors mentioned by him were certainly present in my case, with the exception that the flies did not ingest the parasites along with blood, which he states is essential for them to live and develop in. I find it difficult to understand how a non-bloodsucking fly, such as *Musca nebulo*, can obtain blood from a sore, for the only medium in which I found the parasites discharging from the sore, was a whitish fluid containing leucocytes. Lastly, the fact that the disease never spreads in a house during the fly season, even when several people with sores in a suitable condition are present, is I think very strong evidence against the fly transmision hypothesis. In Cambay the disease is most frequently contracted during the cold weather, which is very severe, the thermometer frequently registering a few degrees above 0°C, and at this time of the year house flies are very scarce.

Cardamatis and Melissidis state that the parasite of Oriental Sore in Greece (?) lives as long as six days in the alimentary tract of the house fly (species not stated), but they do not say whether they used bred flies in their experiments. From the fact that wild house flies are commonly infected with several species of herpetomonads, such experiments as they record are of little or no value.

EXPERIMENT III.

Is the parasite transmitted by lice,—Pediculus vestimenti and Pediculus capitis L. ?

It may be thought that lice are the most suitable carriers of the parasite, more particularly the species found on the body, as they may crawl over a sore, and thus become infected. Later they may be transferred to a susceptible person and infect him. A large number of body and head lice were obtained, the former with great difficulty, they were then kept on small pieces of cloth, in glass tubes, and regularly fed on the case mentioned above ; others were allowed to crawl over the surface of the sore, and to suck up blood from the margin.

Many lice were bred, and then fed from the first instar up to the mature insect. Their alimentary tracts, salivary glands, and other organs, were regularly examined in fresh and in stained preparations, but not a single parasite, either changed or unchanged, was found in any of them. I could therefore come to no other conclusion but that lice (*P. vestimenti* and *P. capitis*) are not the natural invertebrate hosts of the parasite. The statements of the people themselves confirm these results ; educated Indians of high caste, who wash daily and change their clothes often, and who practically never harbour body lice, and very seldom head lice, contract the sore as readily as people who never wash and who harbour such vermin. Further, Europeans who have contracted sores in Cambay, particularly the case of a lady who had one on her lower lip, strongly oppose the idea that lice are the transmitters.

EXPERIMENT IV.

Is the parasite transmitted by any species of mosquito or other biting fly ?

In Cambay *Stegomyia sp.* was extremely common throughout the year ; it is a voracious blood sucker, often biting through clothes. A large number of these mosquitoes were bred out in the laboratory, and fed in test tubes on the case, some on the margin of the sore, and others on distant parts. They were examined at regular intervals, but I was unable to find any parasites, either changed or unchanged, in any of these mosquitoes ; they were not infected with a natural herpetomonas, which in the case of mosquitoes comes through the larvæ. There was then no evidence whatever to shew that this species of *Stegomyia* was the natural carrier.

No other species of mosquito was experimented with chiefly for the reason, that, as I was working single handed, I was unable to collect larvæ in sufficient numbers to carry out experiments, and also for the reason that in Cambay the localised nature of Oriental Sore strongly opposes the hypothesis that a flying insect is the carrier. As I have pointed out above, the railway staff, who live around the station, about a quarter of a mile from the endemic area, never contract the disease. This is a very striking fact, for flying insects such as house flies and mosquitoes, which may have become infected in the town itself, would have every opportunity of biting people living just on the outskirts.

Phlebotomus sp., probably *babu* Annandale, was only seen during the rainy season, June to September, and then in small numbers. I was bitten by a few of these flies but with negative results ; four specimens caught in the Hospital

were examined, but nothing was found in them. During the cold weather, this fly was never seen or felt, although it was carefully searched for in likely places; owing to its painful bite it is well known to the inhabitants, but I could obtain no information which would shew that there were any present during the cold weather when the disease is most frequently contracted.

Philaematomyia insignis Austen, a common cattle fly, was abundant in Cambay throughout the year, but I was unable to obtain any positive information that this fly bites human beings; I have only known it to bite man on three occasions in Madras. It never contains any flagellates.

Stomoxys calcitrans, also common in Cambay, rarely, if ever, bites human beings; it is frequently infected with a natural herpetomonas. No feeding experiments were carried out with either of these flies.

Two non-biting flies, *Musca pattoni*, Austen, and *Musca convexifrons*, Thomson, are common in Cambay, and, as in Madras, they have the remarkable habit of sucking up the blood which exudes from the bites of *Philaematomyia*, *Stomoxys*, *Tabanus*, *Chrysops* and *Haematopota* inflicted on cattle. They were never however seen sucking up the discharge from sores.

In view of the recent work of Basile, that the dog flea *Ctenocephalus canis** is the probable transmitter of Canine and Infantile Kala Azar, and seeing that street dogs were numerous in Cambay I endeavoured to find out whether fleas could have anything to do with the transmission of the parasite. These dogs seldom, if ever, go into the people's houses, but live entirely on the streets, and I never saw or heard of a single one having a true Oriental Sore. Fleas (*Pulex irritans* and *Ctenocephalus felis* were chiefly prevalent during the cold weather, but I was unable to get them in sufficient numbers to carry out feeding experiments and the inhabitants of Cambay could give me no information regarding these fleas as being the probable carriers of the parasite. With regard to fleas, it should be remembered that they are commonly infected with natural flagellates, crithidia and herpetomonads, for Miss Porter has recently recorded a crithidia from the alimentary tract of *Pulex irritans* and I have found a species of herpetomonas in *Ctenocephalus felis*, indistinguishable, both in its preflagellate and flagellate stages, from the parasites of Kala Azar and Oriental Sore. This herpetomonas is found in about 5 per cent. of this flea, and probably also occurs in *C. canis*. It is important to note that the flea acquires the infection in its larval stage and that unless this fact is remembered, it would be very easy, on finding flagellates in an adult flea taken off a dog infected with Kala Azar, to come to the conclusion that they represented stages in the development of the dog parasite. The figures and descriptions given by

* I have it on the authority of the Hon. N. C. Rotschild that there is no such flea as *Ctenocephalus serraticeps*, and that the flea mentioned by Basile is none other than *Ctenocephalus canis*.

Basile exactly coincide with what I have found in *Ctenocephalus felis;* I have examined over 400 cats in Madras and have never found them infected with any such parasite.

EXPERIMENT V.

Is the bed bug Cimex rotundatus the true invertebrate host of the parasite of Oriental Sore?

In order to settle this question beyond dispute, I bred a number of bugs through three generations in Madras and took them with me to Cambay. They were fed regularly during June, July and August on the case mentioned above, some in the vicinity of the sore and others at distant parts. In order that there may be no misunderstanding with regard to my feeding experiments, I wish it to be clearly understood that a bug cannot possibly dislodge the parasites when it inserts its proboscis into the margin of a sore. Its proboscis is not long enough as the parasites are deeply situated, so that the blood it sucks up, can only come from that which is circulating at the spot. Bugs will not feed on the surface of a sore which is discharging parasites. This was definitely settled by feeding bugs at the margin of a sore, and then examining their stomach contents immediately afterwards. The large macrophages or free parasites were never found. Although, in a few of the bugs, unchanged parasites were found, no development was seen in about 250 examined. My attention was next directed to the probable explanation of the failure of the parasites to flagellate in the bug. Early in my investigation I heard of a case of Oriental Sore in a Parsee boy who came to Cambay for the first time in the beginning of December 1909, and who left Cambay about January 5th, 1910. On returning to Bombay he contracted a mild attack of small-pox, and about three months after leaving Cambay, a small red spot was noticed on his cheek. This slowly developed and eventually proved to be a true Oriental Sore; later two other sores formed on his body. There could be no doubt regarding the time of the year when this boy contracted the disease. This at once led me to make a careful enquiry into the histories of those people who had had occasion to come to Cambay for short periods, and it was definitely found that about 95 per cent. of the sores were contracted during the cold season which extends from the middle of October till the end of February. Having ascertained these facts, I at once decided to continue my feeding experiments with bugs and to simulate as near as possible the cold weather conditions; the bugs after being fed, were kept in a small tin box surrounded with ice. These experiments were begun on July 25th, 1910, and on August 8th, on dissecting one of the

bugs, a nymph approaching its last ecdysis, about twenty pairs of actively dividing flagellates were recovered. There could be no doubt as to the origin of these flagellates, for in the first place they were found in a bug which had been bred in Madras and brought to Cambay, and secondly, although I have examined between 2,000 and 3,000 bugs from different parts of India, I have never yet found them to contain flagellates, other than those obtained as a result of feeding experiments.

Having proved that the parasite of Oriental Sore can, and does, reach the stomach of the bug *C. rotundatus* when it is fed on the peripheral blood, and that when such bugs are kept at a suitable temperature (from 22° to 25°C.) the parasite will develop into a flagellate; I decided to feed a large number of bugs on the case, and then to take them down to Bombay and to feed them on suitable people there. Through the kindness of Surgeon-General Bannerman, the then Director of the Bacteriological Laboratory of Parel, Bombay, I was able to feed these bugs on the Laboratory peons. Though they were fed for about a month, and since then a year has elapsed, none of these men developed any sores. I returned to Cambay and fed another series of bugs on the same case and on taking them to Bombay they were fed on other suitable people. These experiments, however, also failed. There were no young nymphs among the bugs used in these experiments, almost all being adults. On returning to Cambay for the second time, I found that the sore was now useless for further experiments as the parasites had disappeared. I was therefore compelled to search for another case, and a suitable one was not found till December 1910. In the mean time I examined a large number of cases but unfortunately none of them were suitable for experimental purposes. The new case had no less than five sores, four situated on the right arm, and one on the dorsum of the left foot: he had already had two the year previously. On puncturing the sores particularly those on the arm, they were found to be extremely rich in parasites, numbers of the polymorphonuclear leucocytes being infected. A large number of bugs were obtained from Bombay and were fed on the boy's right arm, at a distance from the sores, so they could only ingest parasites in the circulating blood. Here again it was unfortunate that, except for one tube of bugs, all the rest were mature insects. I decided to dissect 100 adults and to find out what percentage became infected. The dissections were carried out at intervals from one to five days after the last feed, and I found four bugs containing parasites, some in an unchanged condition still in leucocytes, and all the intermediate stages shewing the formation and extrusion of the flagellum and the multiplication of the resulting flagellates, up to the long mature forms. The remaining bugs, some 200 in number, were taken to Bombay and

fed on a susceptible person ; a few more were dissected, making up the 100, and two were found infected. In one, a nymph approaching its last ecdysis, I found in its midgut in the fresh condition a group of eight round and oval bodies lying against the wall close up to the junction of the œsophagus with the midgut ; there were also two adult flagellates actively dividing. This bug had first fed on the case at Cambay on January 4th and was then probably a nymph in its second instar, and was last fed on January 26th. It was dissected on January 31st, that is, five days after its last feed. On smearing out and staining the midgut, it was found to contain the oval bodies mentioned above. They measured from 4 to 5·5 μ, in length and from 3 to 4·4 μ. in breadth. The nuclei presented a very striking appearance, for their outlines were obscured by small pink granules, many of which were situated at some distance in the protoplasm. It was difficult to locate the blepharoplasts, which although staining in the usual way, were distinctly smaller than those seen in the very similar stage found in the sore in man. The protoplasm did not take the usual blue colour, so characteristic of young flagellating forms, nor were there any appearances suggesting the formation of the flagellum. There could be no mistaking these parasites for young pre-flagellating forms, for they differed in every respect and they were certainly not degenerating. I came to the conclusion that they represented the post-flagellate stages of the parasite. Several other facts strongly support this view ; they were all grouped together, evidently adhering to each other by some sticky substance, an appearance which is characteristic of the post-flagellate stage of the herpetomonads of insects ; they were large, and their nuclei exhibited a peculiar granular appearance which is always seen in the post-flagellate stages of these parasites. Further, they were found in a bug, five days after its last feed, and had they been the pre-flagellating forms they should have exhibited the changes preparatory to the extrusion of the flagellum. In none of the bugs, when they were kept at a suitable temperature, were parasites seen which had failed to flagellate ; this only occurred in bugs kept at a temperature above 25°C. Although I have dissected several thousands of bugs during the last five years I have never once seen any appearances similar to those described above, so that these bodies could be none other than some stage of the parasite of Oriental Sore. The bugs that were fed on this last occasion on a man in Bombay have also failed to produce Oriental Sores ; the nymphs which were fed at Cambay from the first instar, were unfortunately not fed beyond the third or fourth, as I was then under the impression that the adult bug was the most probable transmitter. Before I left Cambay for the last time I decided to inoculate myself from a suitable case, and this was carried out on December 24th, 1910,

being performed subcutaneously with a fine glass pipette on the inner side of my left calf. Ten days following the inoculation nothing was to be seen, but on January 9th a distinct nodule was felt, and on puncturing it, parasites were readily found.

Since returning to Madras, I have fed a large number of bugs on myself, with the object of definitely finding out whether the parasite, after flagellating in the adult bug, is able to live for long periods and to complete its cycle of development; about 500 adult bugs were used in this experiment. The following facts were definitely ascertained and which conclusively prove that the parasite, when ingested by the adult bug and also by the nymph, never flagellates at a temperature above 25°C. If bugs kept at this temperature contain parasites the latter only degenerate. Adult bugs kept at a suitable temperature (22° to 25°C.) and which had ingested parasites, always shewed flagellates, but they were never seen beyond the tenth day, clearly indicating that, in the adult bug, the parasite does not complete its developmental cycle. It was also found that the parasites never multiplied to any great extent, and no multiple segmentation resulting in rosettes was seen. There is one point, however, which requires further study, I have found that by keeping bugs at a temperature of from 18° to 20°C. the blood in their stomachs remains almost unchanged up to 48 hours after it has been ingested, this being even more marked in the nymphal stages. This suggests that the digestive process in the nymph is not nearly as active as in the adult, and it could be readily understood that in an adult bug kept at a high temperature, the parasite, on being freed from a leucocyte, would find itself in a medium unsuitable for the profound changes which take place between the pre-flagellate and the flagellate stages. It is interesting to note that Oriental Sore in India only occurs in those parts where there is a decided cold weather, for instance Karachi, Quetta, Jacobabad, Dera Ismail Khan, Delhi, Lahore and Cambay. As far as I am aware it does not occur anywhere south of Cambay or east of Delhi. While in Cambay I kept accurate thermographic records and studied the temperature recorded at the Hospital for the last 10 years. I found that during the cold weather, which frequently begins in the middle of October and lasts till the end of February, the temperature during the twenty-four hours keeps well below 25°C. I cannot help thinking that there is a factor of fundamental importance underlying the restricted geographical distribution of Oriental Sore in India, and I am convinced that the explanation is to be found in the relation of the temperature to the digestive processes in the bug.

Having now narrowed down the investigation to the nymphal stages of the bug, I decided to waste no more time in dissections, but to feed nymphs on

myself through the first and second instar, and then feed them continuously on a susceptible person, my wife volunteering for that purpose. These experiments were carried out in June, July and August 1911. Three tubes, containing about 100 nymphs, were fed daily, and between the feeds were kept in the cold incubator. Unfortunately, as it was the hottest time of the year in Madras, the thermometer never registering below 28°C. and for the most part being between 30° and 35°C., it was extremely difficult to keep the incubator at a constant temperature, and although large quantities of ice were used, it frequently registered as much as 27°C. The bugs were always fed between 6 and 8 P.M., and for these two hours they were necessarily exposed to a high temperature. Towards the end of July a small spot, simulating the early stage of the sore, developed on one of the places where the bugs were fed. It slowly increased in size until a perceptible nodule could be felt, but since then has not grown any more. An attempt was made to puncture it at the end of August, but owing to its small size, it was impossible to say for certain that the pipette had entered the nodule; nothing was found in the blood drawn up. It is hoped later to puncture it again. Feeding experiments with nymphs will be again started as soon as the cold weather in Madras sets in, and at the same time, a number will be dissected, in order to confirm the observations made above, namely, that the parasite only completes its developmental cycle when ingested by the nymph, and that, if the parasites are still in the early stage when the nymph becomes an adult, they fail to complete their development. In all the dissections that were carried out, both in Cambay, Bombay and Madras, when parasites, particularly flagellates, were found in the midgut of a bug, the other organs, such as the salivary glands, ovaries, œsophagus and pumping organs, were invariably examined, but in not a single instance was I able to find a parasite of any kind outside the alimentary tract. A most careful search was made for them in the hind gut of the bug, but they were never found there; this part of the alimentary tract always contains black granules, the remains of the digested blood, and is a most unsuitable medium for the parasite.

In view of the fact that the parasite flagellates in the bug, and only at a temperature below 25°C., it is necessary to consider shortly any observations which tend to support the hypothesis that *Cimex rotundatus* is the invertebrate host of the parasite of Oriental Sore in Cambay. This bug was extremely prevalent throughout the year and most of the people regularly used bug traps, small pieces of wood with numerous perforations, which they placed in their beds and into which the bugs nearly always crawled after having fed. This practice is universal amongst the Hindus, who form the bulk of the population, Mahomedans periodically shake the bugs out of their beds just outside their

houses. One of the most striking facts in connection with this disease, as I have already mentioned, is that it never tends to spread from one person to another in the same house, during the same season. Over 50 histories exemplifying this fact were carefully studied, and no better example could be given than the following case. An Indian Salt official was transferred for duty to Cambay in March 1909; he was accompanied by his wife, widowed sister and her two sons. The first few days in Cambay they resided in the town, in the house of an old resident, later going into a house which had been unoccupied for a long time. While staying in the friend's house they were severely bitten by bugs. About August the man, his widowed sister, and her elder son contracted the disease almost simultaneously, the wife and the other nephew escaping. The man had two large sores which lasted nearly two years, yet his wife who occupied the same room, and even the same bed, did not develop Oriental Sore till a year later, and the little nephew at the same time; the latter case I had the opportunity of examining in its early stage. Numbers of cases were studied in which there was one child who had Oriental Sore in a house and several others who did not contract the disease till years afterwards. This peculiarity in the spread of the disease cannot be overlooked, and in my opinion it points very clearly to the transmission of the parasite by a blood sucking insect, in which development of the parasite takes place slowly, and that once that insect becomes infective, it may remain so for a long period, thus being able to infect several people or the same person in more than one place. It also suggests that the insect in question is usually transferred from one house to another before it is infective. The last case studied in Cambay had four sores of the same age on his right arm, three of which were on the inner side of the forearm, about three inches apart. This fact suggested that they were acquired at the same time; it is necessary then to consider what blood sucking insect will bite three adjacent parts of the body consecutively. The only likely insects are mosquitoes and the bug. It is true that when a mosquito is disturbed in the act of biting, it may quite possibly alight close to the spot where it was first feeding, and that if disturbed again it may return to another adjacent spot a few inches away from the former ones. Any one who has observed the habits of mosquitoes will, I think, agree with me that the chances of this happening are small; most mosquitoes when disturbed fly a considerable distance, and when they again alight, select a more distant part of the body. The only mosquitoes, as far as I am aware, which do return to the same place are those belonging to the genus *Stegomyia;* I frequently observed this habit exemplified in the case of the species prevalent in Cambay, and this was one of the reasons why it was carefully studied. As far as the parasite of Oriental

Sore in Cambay is concerned I failed to find any other fact which pointed to this mosquito as being the transmitter.

The bug, whose habits I have now observed for nearly six years, is the blood-sucking insect which always returns either to the same spot or to one close by; it also has the peculiar habit, and one which, as far as I know, has not yet been recorded, of returning to feed after it is replete with blood. On watching such an insect, one cannot help wondering how it can possibly ingest any more; nymphs, as well as adults, exhibit this habit. A number of cases in Cambay were found to have either scars or sores close together on the same part of the body, shewing that this method of multiple infection frequently takes place.

The Hindoos in Cambay regularly empty their bug traps into the street, and the bugs naturally crawl into an adjacent house. Those who do not use bug traps periodically clear out bugs from their houses by shaking them out of beds and mats into the street. I calculated from the single observation of the nymph which contained the post-flagellate stages, that the cycle of development averages about twenty-two days. It can then be readily understood that a nymph, which had ingested a parasite but had not yet become infective, may be transferred to another house, thus explaining why the disease does not spread in the same house even though there are a number of suitable cases. There are several other facts which suggest the bug as the probable transmitter. I have already pointed out that only a small percentage of the cases are suitable for the transmission of the parasite by the peripheral blood, for the simple reason that the parasitic area in a sore soon breaks down, so that there are extremely few, or no parasites left a short time after the sore has reached its maximum size. It therefore follows that there is only a brief period during which the parasites can possibly pass into the circulating blood, and then only in small numbers. It is obvious that a blood-sucking insect, which is constantly present, and sucks the blood of the same person on many consecutive nights is much more likely to ingest a parasite, than a flying insect such as a mosquito or a phlebotomus, which hardly ever sucks the blood of the same person on more than two or three occasions. Such flies must necessarily soon leave a house in order to lay their eggs. Recently Miss Porter related to me the case of a young European school girl who contracted the disease at Bangalore where Oriental Sore is not endemic. One night a fire broke out in the school, and the children were hurriedly removed to neighbouring houses. This girl was taken to a native house, the inhabitants of which had recently come from Cambay. She woke up later in the night and found a bug which she stated was a "baby" one biting her on the left forearm. She killed it and

remembered seeing the small red puncture next morning, but soon forgot all about it. Three months later a small pimple was noticed on the same spot which gradually developed into a typical Oriental Sore, which the people in whose house she had slept on the night of the fire, had no hesitation in calling a "Cambay boil." The sore lasted many months resulting in a typical scar, which was recently excised in England on account of the disfigurement. I have elsewhere drawn attention to a case recorded by Schneider in a European lady who lived for some time at Zerguendeh, a village close to Teheran, who on waking one night with the sensation of something biting her on the cheek, put up her hand and caught a bug. Schneider adds that in the same house there were four children who had had the sore the preceding year, and that the house was infested with bugs. The lady subsequently developed a large sore on the spot were the bug had bitten her.

Lieutenant-Colonel Ashton Street, I.M.S., in the discussion on Dr. Row's recent paper, relates the case of a military officer who developed several Oriental Sores in Karachi a few days after being pestered by bugs in the hotel in which he was staying. Dr. Row, in reply, takes exception to the short incubation period, and states that it is always of about two months' duration in the monkey. Here again my studies of the disease in monkeys *Macacus sinicus* are at variance with those of Dr. Row. I have reproduced, by subcutaneous inoculation, a typical lesion in a monkey after eighteen days' incubation, and my own sore developed on the sixteenth day ; in both cases the parasites were recovered on these days. It will be seen then that my observations correspond exactly with those of Nicolle in Tunis. The reason why Dr. Row finds that the incubation period in his monkeys is nearly always two months, is very simply explained. I had the opportunity, when he visited Madras, of seeing the technique he employed. He first scarified the sore with a knife, then pipetted the fluid up into a capillary tube and deposited it on similar scarifications and rubbed it in ; the operation lasting about ten minutes. On the contrary I puncture the sore with a fine capillary pipette and draw up the infected material, and immediately with the same pipette, puncture the skin of the monkey and blow the fluid in. This operation lasts a few seconds. In this way I have found that, by inoculating a monkey with a very few parasites, the incubation period is about two months, whereas if richly infected material is used, containing many thousands of parasites, the early nodule may be detected on the fourteenth day. On puncturing such a nodule, the parasites will be found in the macrophages, and cannot therefore represent those which were originally inoculated into the skin, for in the interval nothing can be felt. This method of reproducing the disease is exactly comparable to the way in

which it is produced in nature, that is, the parasite is deposited into a healthy bit of skin. I believe the variable incubation period, one week to six months as seen in those people who came to Cambay for short periods and then went away, is easily explained by the smaller or larger number of parasites inoculated into the skin; it can be well understood that the degree of infection of a blood-sucking insect, such as the bug, may vary, so that in one case many parasites may be inoculated and in others very few.

The cases I have recorded above are of considerable importance as the majority of people who have become infected with Oriental Sore are unable to recall definitely whether they have been bitten by any insect.

Lastly when in Cambay, I examined the bodies of several hundred children, carefully noting the site of the scars, and found that they were present on every region of the body, except the genital organs, palms of the hands and soles of the feet. One typical fresh sore, containing numerous parasites, was seen on the scalp, just over the centre of the right parietal bone; this was in a small Mahomedan boy whose head had not been shaved the year previously, as the father who was an intelligent man informed me. Four years before another of his children had had the sore, but this little boy had then escaped. I also saw several scars on the scalps of other children. All observers who have studied Oriental Sore, have repeatedly stated that, as it usually occurs on exposed parts, it cannot be transmitted by the bed bug, because this insect is said to bite only the covered parts of the body. The statement regarding this habit of the bug is totally erroneous, as any one who is willing to take the trouble, can demonstrate for himself. I have a vivid recollection of once sleeping in a bed which contained many of these insects. I was wearing a pyjama suit; the uncovered parts of my body were the lower thirds of my legs, and forearms, my neck and head, and these were the exact places where I was bitten. Indians are accustomed to sleep with only a loose loin cloth on, and to cover themselves with sheets or blankets; thus the whole of their bodies, except this part, are obviously exposed to the bites of bugs. The bug is the only insect (with the exception of the head louse) which will bite the scalp, and one of the commonest places to find bugs is in the head-gear of an Indian; these turbans on being removed at night are usually placed on a peg on the wall, and bugs will regularly crawl into them and hide in the folds. I have repeatedly removed large numbers of these insects from these places. In the same way the topees of Europeans may become infected. This is the only way I can explain the occurrence of Oriental Sore on the scalp. I have now endeavoured to explain why I consider the bug to be the most probable transmitter, and I am well aware of the oft repeated statement that if it were the bed bug, the disease

would tend to spread outside the town of Cambay, for bugs are certainly just as prevalent in the villages round as they are in the town itself. This argument, however, would apply not only to the bug, but to every other blood-sucking insect, especially to winged forms. My own view is, that in this particular disease, we are dealing with a special parasite, whose life history, outside the human body, is governed by very definite factors such as temperature, presence or absence of the parasite in the circulating blood, and the bug in a suitable stage.

It is much to be regretted that such a careful observer as Dr. Row, still endeavours to prove a hypothesis which has only a single fact to support it, namely, the readiness with which the parasite can be scratched into the skin. He ignores the overwhelming evidence in support of the parasite being transmitted by a blood-sucking insect, and has therefore never studied the problem from this point of view.

From my own observations, and particularly the experiments carried out in Cambay, I have no hesitation in saying that the house fly *Musca nebulo* and *Musca sp.* play no part whatever in the transmission of the disease in Cambay, and that Dr. Row's feeding experiments, conducted as they were in Bombay with "trained" flies fed on richly infected material, are in no way comparable to experiments carried out in the endemic area. Although I have failed up to the present to actually transmit the parasite by the bed bug, I have brought forward very strong evidence, by shewing that the parasite only flagellates in the bug below a certain temperature, and that this observation exactly coincides with the geographical distribution of the disease in India. I hope in time to be able to transmit the disease through the bug. I, however, wish it to be clearly understood that the statements I have made above are at present only applicable to Oriental Sore in Cambay. I am well aware that many competent observers have stated that in other places where the disease is extremely prevalent, bugs, either *Cimex rotundatus* or *Cimex lectularius*, are rare. I can express no opinion as to how the parasite is transmitted in these places, but I fully realize that it may have more than one invertebrate host. In Cambay, I have no doubt whatever that the bug *Cimex rotundatus* is the only insect transmitter of the disease. My conclusions have not been arrived at hastily, but are the result of a most careful and exhaustive study of the disease and its probable transmitters.

In conclusion I would like to take this opportunity of thanking His Highness Mirza Jafir Ali Khan, Nawab of Cambay, and Mr. Madhavram Harinarayan, the Dewan of Cambay, for their kindness in making all arrangements for my comfort during my stay in Cambay; also Mr. Dhanjiboy, the Chief

Medical Officer of the State, without whose help and experience, I could never have accomplished the difficult task I had to undertake. One of the greatest obstacles I had to contend with in Cambay was the procuring of suitable cases, and it was here that Mr. Dhanjiboy's influence was most valuable.

REFERENCES TO LITERATURE

PATTON, W. S.—"Preliminary Report on the Development of the Leishman-Donovan Body in the Bed Bug." Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India. New series No. 27. The Development of the Leishman-Donovan parasite in *Cimex rotundatus*. Scientific Memoir No. 31.

ROW, R.—" Further Observations on Leishmania Tropica of Oriental Sore of Cambay, India." Journal of British Medical Association, page 867, September 24th, 1910.

" " Leishmania Tropica and the Oriental Sore of Cambay." Proceedings of the Bombay branch of the British Medical Association, January 29th, 1911.

SERIZIAT.—" Etudes sur l'oasis de Biskra," Paris. (Cited by Hirsch, Geogr. Pathol. III, page 477.)

LAVERAN, A.—" Contribution a l'etude de bouton de Biskra." Ann. de Dermatologie, 2 S. I., 173—197.

CARDAMATIS, J. P.—" Role probable de la mouche domestique et dans la transmission des
AND *Leishmania*." Bulletin de la Societe Path. Exot. Tome IV,
MELISSIDIS, A. No. 7, page 459, Juillet 1911.

BASILE, C.—" Sulla Leishmaniosi et sul suo modo di Transmissione." " *Rendiconti della R. Accademia dei Lincei*." T. XIX, Nov. 20th, 1910, pp. 523—527; T. XX, January 8th, 1911, pp. 50-51; T. XX. February, 19th, 1911, pp. 50-51 and T. XX, March, 1911, pp. 479—485.

PORTER, A.—" Further Remarks on the Genera Crithidia; Herpetomonas, and Trypanosoma and Dr. Woodcock's views thereon." Parasitology, Vol. IV, No. 2, July 18th, 1911.

NICOLLE, C., et MANCEAUX, L.—" Recherches sur le Bouton d'Orient cultures, Reproduction experimentale, Immunisation." Annales de L'Institut Pasteur, September 1910, pp. 673—720.

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